Semi Automated Rack Collection of Brachyuran Crab Zoea in a Spawner Unit

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ABSTRACT

The design and operation of a small research scale berried female rearing system for the collection of brachyuran first formed zoea in high quality and quantity are described in the present paper. The envisaged novel zoea collection system discloses a tray shelf system with an aqua terrarium on the upper shelf with provision of an accessible dry zone for the crab facilitating movement of between the body of water and the terrarium gravel pile. As larval aggregation in the bottom of the tanks will certainly cause ‘‘tangling’’, damage larval appendages or even the death of the larvae, this immediate auto separation through a photoexposure regime design holds promise.

تجميع يرقات سرطان البحر باستخدام وحدة تفريخ نصف آلية

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المُستلخص

تم في هذه الدراسة وصف التصميم وعملية التشغيل لنظام تجريبي لعملية تجميع يرقات السرطان الحقيقي في المرحلة الاولى بعد الفقس وبأعداد كبيرة وبنوعية عالية. من الجدير بالتنويه أن إن نظام تجميع اليرقات هو عبارة عن حوض برمائي يعتمد على رفين. الرف الأعلى منه به جزء يابس يتألف من كومة من الحصى يسمح للحيوان البرمائي للتحرك بين الجزء اليابس، فيما الجزء الآخر الأسفل من الحوض فهو جزء مائي. وبما ان تجمع اليرقات في قاع الحوض المائي يتسبب في تشابك يرقات سرطان البحراو الإضرار بزوا ئد اليرقات او حتى الى نفوقها، عليه فأن طريقة الفصل الآلي المباشر، والتي هي محور هذه الدراسة، من خلال التعريض الضوئي لليرقات بعد فقسها من خلال التصميم المقترح يعد نظاماً واعداً.

Introduction

Despite the zoo technical breakthroughs that have been achieved in the recent past on the larviculture of marine brachyurans of the Red Sea, feasible commercial scale protocols for the quality mass separation of the hatched zoea are still missing. Quality of newly hatched larvae or their inherent viability is regarded as a significant factor influencing the success of hatchery production. Damaged larvae are also more exposed to the action of opportunistic pathogens, a major constraint to the larviculture of decapods larvae (Smith, *et, al*., 2003); and (Bourne, *et, al*., 2004& 2007). Elevated levels of abnormal or non-viable eggs or larvae are considered undesirable characteristics (Millamena, *et al*., 1999). The system discussed here encompasses brood stock conditioning, egg incubation and spawning, larval separation and conditioning effects.

Materials and Methods

The design has been illustrated in pictorial form in figure 1.

FIG. 1. AQUATERRARIUM

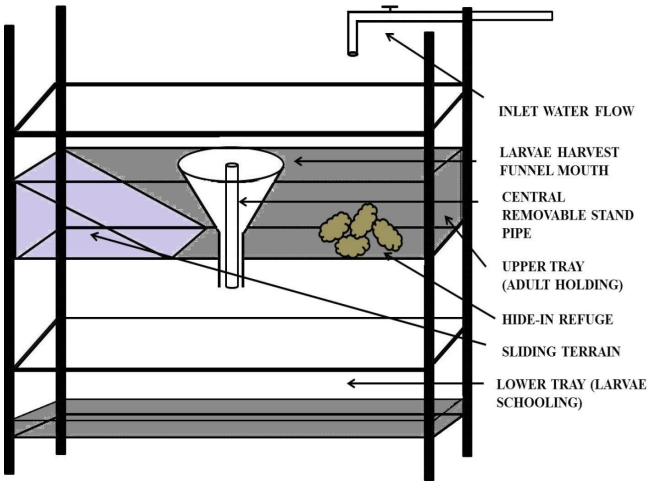

Figure 1: Aquaterrarium

Upper tray enclosure is an aquaterrarium (painted black on the lower-half lateral side-walls and transparent upper-half side walls with translucent tray floor-bottom) and partially filled with water on one rectangular extreme and a large amount of gravel piled higher than the water level at the other extreme side. There is a standpipe at the centre of the water body with a wide open circular funnel that fits the bottom vent hole and prevents the water body from draining to the lower tray enclosure. Located above the upper tray enclosure is a dispensing head reservoir container that drips seawater in need through a ball-valve. Rising water column is limited by the funnel stand pipe and drain-pooling the water down into the funnel and straight down into the lower tray enclosure. Polyacrylic sheet with abundant 5 mm holes cover the upper tray enclosure. A planar up schooling process for zoea is effected in response to the actively thin planar light radiation subjected to the top surface plane of the tray. The most active larvae (the ones displaying pronounced positive phototactic responses—approximately 90%) were up schooled and transferred to the rearing tanks.

The set up was maintained at room temperature 28±1°C with a dark-light cycle of 12:12 h. The system was maintained at 30ppt salinity. Upper tray has exclusive temperature control with glass sealed immersion auto control heaters at 32°C unlike the lower tray which registers room temperature. Biological feeds are usually kept on the gravel dry sloping terrain of the upper tray by partially draining the water column for 12 hours in the day. Sloping terrain is bath-immersed at night for 12 hours after prior removal of the fresh biofeeds. Vertically half-cut vinyl chloride pipes of 10cm in diameter and 30cm in length were provided as shelters for the ovigerous females. Pebble mound served as a light-shunning refuge for the females. Dihydrostreptomycine sulfate was added to the rearing water at 5ppm to prevent bacterial attachment on the larvae. To thwart sources of fungal infection from parents in the upper tray to spawning progenies, trifluralin is administered at 0.0015mg 1^{-1} level.

First formed zoea 1 separation process starts immediately after spawn-release. This is to facilitate lesser detention time and contact with the spawning bio protein debris and incubative bacterial proliferation. The phenomena of phototactic up schooling of zoea is in obedience to the planar photons of light illumination. By the input water inflow to the upper tray, auto up-collection of zoea conducts to the funnel in the course of water flow and eventually drains down to the lower tray fluid mesocosm).

To estimate fecundity and hatching rate, the total number of zoea larvae produced was estimated from three 100 mL aliquot water samples taken from the hatching tank. The newly hatched zoea and un-hatched eggs were counted from the sample, and fecundity and hatching rate were calculated using the formula HE = HPZ/Fx 100, Fecundity = HPZ + UHE where F = fecundity, HE = hatching rate, HPZ = total number of the newly hatched pre-zoea, and UHE = total number of unhatched eggs (Arshad, et al., 2006); (Oniam and Taparhudee, 2010).

Results and Discussion

Comparative spawning and zoeal separation studies with the fabricated system design and conventional tank culture system showed contrasting difference in performance values (Table 1).

Table 1: Hatching Performance of *Grapsus lbolineatus* Females in the Aquaterrarium

Performance Factors		I	II	III	IV	V	Mean
Unhatched Eggs (UHE) %	TT	53	48	59	44	42	49.2
	AST	14	27	32	33	37	28.6
Hatched Pre Zoea (HPZ) %	TT	47	52	41	56	58	50.8
	AST	86	73	68	56	63	69.2
Fecundity	TT	4800	3600	2800	3500	5300	4000
	AST	3500	2600	4400	5800	3700	4000
Moult-death Syndrome Cases (on day 7) %	TT	5	7	4	6	5	5.4
	AST	1	3	2	2	5	2.6

(TT.= *Traditional Tank/* AST= *Aquaterrarium Spawning Trials*)

Mean values of percentage unhatched eggs were 49.2 and 28.6 in the traditional tank (TT) and Aquaterrarium tank (AT) respectively; Mean percentage hatched pre-zoea were 50.8 and 69.2 in traditional tank (TT) and Aquaterrarium tank (AT) design entities; Mean fecundity was 4000 and 4000 in the (TT) and (AT) models. Mean percentage moult-death syndrome cases were 5.4 and 2.6 in (TT) and (AT) systems respectively. Mortalities were suspected to have been caused by presence of bacteria in the rearing water (Blackshaw, *et al.,* 1999). Hatch success rate is dependent on a variety of factors - parasites and diseases ; parental behavior (Fernandez, *et al.,* 2000). To obtain a high hatching rate therefore requires that the female crab be maintained under optimal water quality and environmental conditions. Adult female maintenance systems vary widely between studies, ranging from flow-through systems (Du Plessis, 1971), to re-circulating systems (Heasman and Fielder 1983); (Mann, *et al.,* 1999) and stagnant systems with a complete daily water exchange (Djunaidah, *et al.,* 2001). (Bourbon, 2005) devised a bi level aquaterrarium for crab – a first level for placement proximate to the top of an aquarium tank and providing an orifice for the crabs to enter and exit the aquaterrarium ; and a second level which is affixed to the first level for providing a dry area on which the crab can be situated. (Bourbon, 2005) improvised the system addressing the problems of maintaining adult crabs and tropical fish in the same environment.

In conventional spawning systems in aeration-supported containers, mycosis induced poor viability of larvae due to prolonged co habitation time for the mother and the hatched zoeal lot. To combat this microbial load-aided survival decimating factor and reduce the co residence time , this system design helps for the effective sanitation and husbandry procedures in the upper tray and instant expulsion of zoea to the bottom tray. (Armstrong, *et al*., 1976) reported effective control of mycosis with trifluralin at 0.0015 $mg1^{-1}$. With trifluralin, more than 80% of hatching pre zoea always successfully molted into the first zoeal stages (Caldwell, 1977). Larval molting also improved in the present study with less moult-death syndrome cases on day 7, while administering similar trifluralin doses (in the lower tray) and lower pre-hatching salinity of 30 ppt (in the upper tray) with the fabricated system design. *Chasmagnathus granulata* showed intrapopulational variability in the biomass of eggs and freshly hatched larvae. Salinity at egg laying appeared to influence initial egg size and biomass (larger at low salinities). This suggests both a plastic response (higher yolk reserves) and passive physical changes (higher water uptake) at lower salinities. Total embryonic development time to hatching was not affected by salinity. Initial larval biomass depended on initial egg biomass and on the C and N losses occurring during embryonic development. Since other experiments showed that survival is positively correlated with initial larval biomass (Gimenez, 2000), variability in initial egg biomass and effects of salinity may be important for the subsequent survival of early larvae in the field (Gimenez and Anger, 2001).

Egg masses of laboratory-maintained crabs frequently normally get contaminated by bacteria, fungi, filamentous bacteria, ciliate protozoans etc and therefore, bacterial suppression by exclusion or dilution is mandatory. As the presence of lipids reduces sea water surface tension and facilitates bubble coalescence (Guyon, *et al*., 2001), thereby decreasing gas holdup, biological feeds like squid and gravid oyster meat present in the upper tray can exert lipidic films on the spawned zoea and accelerate bacterial proliferation, but the lipidic slime is diluted by the immediate separation of the zoea and recirculative air-lift effects in the lower tray to expel oily bio films. This concept design is a radical change from conventional designs. It is known that opportunistic pathogenic bacteria may be a major cause of decapod larval mortality, particularly among those

displaying long larval development (Bourne, *et al*., 2007). Unlike static culture systems, a flow through dual-tray spawner unit handles microbial load at ease by flushing and or dilution thereby promoting hatchability and survivorship of zoea.

Conclusion

Chief factors of merit are the application of hygienic quarantine protocols under controlled space limits ; clean recirculation of zoea rearing system ; temperature assisted bacterial incubation critical control for the larvae rearing tray excluding the berried female tray; dual stage provision of aquaterrarium substrates and instant facilitation of an alternative residence for pre zoea upon eventual spawning. This system is suitable for breeding programs in which water needs to be heated to 32^{o} for spawning (in the upper tray) and cooled in advance for post-spawn rearing(in the lower tray). The problem of injury to larvae while separating them which easily occurs in the traditional breeding system is avoided in this system. The risk of losing larvae due to the potential adhesion to the thin nitex scooping mesh is avoided. The risks of contamination are certainly magnified if cultured larvae display damaged appendages. This design has direct application potential in shift-spacing broodstock in an aquaterrarium complex, conditioning the fecundity and zoeal quality and overall survival of brachyuran breeding in captivity.

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